

Amendments to the Specification:

Please amend the title as follows:

"Lrp4/Corin DOPAMINERGIC NEURON PROLIFERATIVE PROGENITOR CELL
MARKERS Lrp4/CORIN DOPAMINE PRODUCING NEURON PROLIFERATION
PRECURSOR CELL MARKER"

Please amend the paragraph on page 4, line 36 through page 6, line 6, beginning,
"More specifically, the present invention relates to:..." as follows:

--More specifically, the present invention relates to:

[1] a dopaminergic neuron proliferative progenitor cell marker polynucleotide probe comprising a sequence selected from the following nucleotide sequences

(1) to (5):

(1) a nucleotide sequence complementary to a nucleotide sequence of SEQ ID NO: 1 or 2;

(2) a nucleotide sequence complementary to a nucleotide sequence encoding an amino acid sequence of SEQ ID NO: 3 or 4;

(3) a nucleotide sequence complementary to a nucleotide sequence encoding a sequence lacking a transmembrane domain in an amino acid sequence of SEQ ID NO: 3 or 4;

(4) a nucleotide sequence that hybridizes under stringent conditions with a polynucleotide consisting of a nucleotide sequence of SEQ ID NO: 1 or 2; and,

(5) a nucleotide sequence comprising at least 15 contiguous nucleotides selected from sequences of (1) to (4),

[2] an antibody against a polypeptide selected from the following (1) to (6):

(1) a polypeptide encoded by a nucleotide sequence of SEQ ID NO: 1 or 2;

(2) a polypeptide comprising an amino acid sequence of SEQ ID NO: 3 or 4;

(3) a polypeptide comprising an amino acid sequence lacking a transmembrane domain in an amino acid sequence of SEQ ID NO: 3 or 4;

- (4) a polypeptide comprising an amino acid sequence with a deletion, insertion, substitution, or addition of one or more amino acids in an amino acid sequence of SEQ ID NO: 3 or 4;
- (5) a polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions with a sequence complementary to a nucleotide sequence of SEQ ID NO: 1 or 2; and,
- (6) a polypeptide that is a fragment of a polypeptide of (1) to (5) comprising at least 8 amino acid residues,
- [3] a method of selecting a dopaminergic neuron proliferative progenitor cell, wherein the method comprises the step of contacting the polynucleotide of [1] with a cell sample thought to comprise a dopaminergic neuron proliferative progenitor cell,
- [4] a method of selecting a dopaminergic neuron proliferative progenitor cell, wherein the method comprises the step of contacting the antibody of [2] with a cell sample thought to comprise a dopaminergic neuron proliferative progenitor cell,
- [5] a method of selecting a postmitotic dopaminergic neuron proliferative progenitor cell comprising the steps of:
 - (1) selecting a dopaminergic neuron proliferative progenitor cell using the method of selecting a dopaminergic neuron progenitor cell of [3] or [4];
 - (2) culturing the proliferative progenitor cell selected in (1); and,
 - (3) screening the progenitor cell cultured in (2) using a postmitotic dopaminergic neuron marker,
- [6] a dopaminergic neuron proliferative progenitor cell prior to cell cycle exit selected using the method of [3] or [4] ~~any one of [3] to [5]~~,
- [7] a postmitotic dopaminergic neuron progenitor cell selected using the method of [5],
- [8] ~~[7]~~ a method of isolating a gene specific to a dopaminergic neuron proliferative progenitor cell and a gene specific to each maturation stage of the

progenitor cell differentiating into a dopaminergic neuron, wherein the method comprises the step of detecting and isolating a gene specifically expressed in the proliferative progenitor cell of [6], or a cell differentiated, induced, or proliferated from the progenitor cell, and
[9] [8] a method of screening using maturation as an index, wherein the method comprises the steps of contacting a test substance with the proliferative progenitor cell of [6], and detecting the differentiation or proliferation of the progenitor cell induced by the contact.--

Please amend the paragraph on page 18, lines 7-22, beginning, "In addition, the present invention provides a method of selecting dopaminergic neurons..." as follows:

--In addition, the present invention provides a method of selecting dopaminergic neurons comprising a step of contacting an antibody for selecting dopaminergic neuron proliferative progenitor cells of the present invention with a cell sample containing potential dopaminergic neuron proliferative progenitor cells. Namely, cells expressing Lrp4 polypeptide, or in other words, dopaminergic neuron proliferative progenitor cells prior to cell cycle exit, can be acquired by contacting a cell sample containing potential dopaminergic neuron proliferative progenitor cells with an antibody of the present invention, and selecting those cells that have bound to the antibody (see Fig. 6). The antibody may also be immobilized on a suitable support, prior to cellular contact. Alternatively, cells that bind with the antibody can be selectively recovered, by contacting cells with an antibody and allowing them to bind, and purifying the antibody by affinity chromatography. For example, if an antibody of the present invention is conjugated to biotin, it can be purified on a plate or column bound with avidin or streptoavidin. In addition, magnetic particles can be bound to an antibody, for example, and the antibody and cells that express on their surfaces Lrp4 bound to the antibody, can be recovered using a magnet. Dopaminergic neurons neuron proliferative progenitor cells that express Lrp4 can be selected by

flow cytometry using a cell sorter and fluorescent-labeled anti-Lrp4 antibodies and such.--

Please amend the paragraph on page 19, lines 1-11, beginning, "In addition, Lrp4-expressing dopaminergic neuron proliferative progenitor cells and..." as follows:

--In addition, Lrp4-expressing dopaminergic neuron proliferative progenitor cells and 65B13-expressing dopaminergic neuron precursor cells can also be selected and/or screened using promoters for Lrp4 and 65B13, respectively (see, for example, Unexamined Published Japanese Patent Application No. 2002-51775). For example, a vector harboring a construct that comprises a gene encoding a detection marker, such as GFP, linked to a promoter region obtained from analyzing the Lrp4 expression regulatory regions to be described later, can be transfected into cells. In addition, a gene encoding a marker can also be knocked in at the Lrp4 gene locus. In either case, specific cells can be selected by detecting the expression of a marker gene specific for dopaminergic neuron progenitor cells. With respect to 65B13, screening can also be performed in a similar manner to Lrp4. For example, the sequence disclosed in Japanese Patent Application No. 2002-307573 can be referred to for 65B13.--

Please amend the paragraph on page 19, lines 12-28, beginning, "The cell sample used herein preferably comprises cells of the ventral midbrain region or..." as follows:

--The cell sample used herein preferably comprises cells of the ventral midbrain region or ~~culture medium~~ cultured cells containing *in vitro* differentiated dopaminergic neurons. *In vitro* differentiation of dopaminergic neurons can be carried out by known methods using cells, such as known ES cells, bone marrow interstitial cells, immortalized neuron-derived cell lines (Published Japanese Translation of International Publication No. Hei 8-509215; Published Japanese Translation of International Publication No. Hei 11-506930; Published Japanese Translation of International Publication No. 2002-522070), or primordial neuron cells (Published Japanese Translation of International Publication No. Hei 11-

509729), as the starting material. Normally, dopaminergic neurons can be differentiated by co-culturing a tissue obtained from a dopaminergic neuron region of the brain, with a sustentacular cell layer derived from neural tissues. Moreover, methods are also known for deriving dopaminergic cells from neural tissues that normally do not produce dopamine, such as the striatum and cortex (Published Japanese Translation of International Publication No. Hei 10-509319). In addition, culturing under hypoxic conditions has been reported to produce cells containing a greater number of dopaminergic neurons (Published Japanese Translation of International Publication No. 2002-530068). A cell sample used in the selection of dopaminergic neuron progenitor cells of the present invention may be a cell population isolated or cultured by any method including the above-described methods.--

Please amend the paragraph on page 21, line 31 through page 22, line 8, beginning, "The efficiency of gene expression screening can be improved by using a DNA chip...." as follows:

--The efficiency of gene expression screening can be improved by using a DNA chip. Here, a DNA chip refers to a miniature array, in which oligonucleotides, DNA clones, or such, are immobilized at a high density on a support surface, such as glass. For example, in order to carry out multiple expression screening, cDNA clones for each gene of interest, or oligonucleotides specific to each gene, are immobilized on a chip to produce a microarray. Next, RNAs are prepared from ~~dopamine-specific~~ dopaminergic neuron proliferative progenitor cells of the present invention, or cells differentiated, induced, or proliferated therefrom, and treated with reverse transcriptase to yield cDNAs. Next, the resulting cDNA sample is labeled with fluorescent tags or other tags, and then hybridized to the microarray. As a result, genes that are actively expressed in the cells have a higher percentage of the total labeled cDNA, while genes that are not significantly expressed have a lower percentage. Namely, the

fluorescent signal intensity which represents hybridization between a labeled cDNA and a cDNA clone or an oligonucleotide on the chip, reflects the expression level of each sequence in the labeled cDNA, and thereby enables the quantification of gene expression.--

Please amend the heading on page 24, line 21 as follows:

--<Analysis of Lrp4 Expression Regulatory Region>--

Please amend the paragraph on page 25, lines 7-9, beginning, "The expression region of the Lrp4 gene isolated in this manner can be used to produce a..." as follows:

--The expression regulatory region of the Lrp4 gene isolated in this manner can be used to produce a protein of interest specifically in dopaminergic neuron proliferative progenitor cells prior to cell cycle exit *in vivo*.--

Please cancel the present "SEQUENCE LISTING", pages 1/35-35/35, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 16, at the end of the application.